

Applicants: Soderlund *et al.*

Serial No.: 08/465,322

Filed: June 5, 1995

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51. [Four times amended] A reagent kit for detecting the presence of a specific nucleotide at predetermined position in a target nucleic acid polymer, comprising: [a target nucleic acid polymer]; a polymerizing agent; at least one deoxynucleotide and at least one chain terminating nucleotide analogue [a labeled nucleotide complementary to a specific nucleotide at a predetermined position in the target nucleic acid polymer]; and an oligonucleotide detection primer having a nucleotide sequence complementary to and capable of hybridizing to a region in the target nucleic acid polymer flanking the 3' end of a [the] predetermined position, such that the oligonucleotide detection primer, when hybridized to the target nucleic acid polymer, forms an oligonucleotide detection primer extension product by an enzyme catalyzed chain extension nucleic acid polymerization [that adds to the oligonucleotide primer the labelled nucleotide complementary to the specific nucleotide at the predetermined position in the target nucleic acid polymer in the presence of the polymerizing agent].

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52. [Twice Amended] A reagent kit according to Claim 51, wherein the oligonucleotide detection primer comprises an attachment moiety.

53. [Twice Amended] A reagent kit according to Claim 51, wherein the oligonucleotide detection primer has a length of from 10-40 nucleotide residues.

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56. [Twice Amended] A reagent kit according to Claim 51 having the detection primer sequence 5' -GTA CTG CAC CAG GCG GCC GC-3'.

57. [Twice Amended] A reagent kit according to Claim 51 having the detection primer sequence 5'-GGC CTG GTA CAC TGC CAG GC-3'.

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59. [Twice Amended] A reagent kit according to Claim 51 having the detection primer sequence 5' -CAG TAA CGG CAG GCG GCC GC-3'.

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62. [Twice Amended] A reagent kit according to Claim 51 having the detection primer sequence 5' -AAC TTG TGG TAG TTG GAG CT-3'.

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67. [Amended] A reagent kit according to Claim 51 wherein the oligonucleotide primer extension product is immobilized to a solid support.

70. A reagent kit according to claim 51, wherein at least one deoxynucleotide comprises a detectable label.

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71. A reagent kit according to claim 51, wherein at least one chain terminating nucleotide analogue comprises a detectable label.

72. A reagent kit according to claim 51, wherein the chain terminating nucleotide analogue is a dideoxyribonucleotide triphosphate selected from group consisting of ddATP, ddGTP, ddCTP and ddTTP.

73. A reagent kit according to claim 51, wherein the reagent kit comprises at least two deoxynucleotides each comprising a different base and at least one of which comprises a label.

74. A reagent kit according to claim 51, wherein the deoxynucleotides are deoxynucleoside triphosphates selected from the group consisting of dATP, dGTP, dCTP and dTTP.

75. A reagent kit according to claim 51, wherein the detection primer hybridizes to the target nucleic acid immediately adjacent the specific nucleotide to be detected.

76. A method for determining the identity of a specific nucleotide at a defined sites in a target nucleic acid polymer, comprising the steps of:

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- (a) providing a detectable amount of a target nucleic acid polymer in a single stranded form;
- (b) hybridizing the detectable amount of the nucleic acid polymer with an oligonucleotide primer, wherein the primer has a nucleotide sequence that is complementary to a sequence in the target nucleic acid polymer, such that when the primer is hybridized to the target nucleic acid polymer the 3' end of the primer binds to a nucleotide flanking the specific nucleotide at the defined site in the target nucleic acid;
- (c) exposing the hybridized nucleic acid polymer to a polymerization agent in a mixture containing at least one deoxynucleotide and a chain terminating nucleotide analogue, such that a detectable primer extension product comprising a labeling moiety is formed if the chain terminating nucleotide analogue is not complementary to the defined site, and
- (d) analyzing the polymerization mixture of step (c) for the presence or absence of the primer extension product containing the labeling moiety at the 3' end thereof, whereby the identity of the specific nucleotide at the defined site is determined.
77. A method according to claim 76, wherein the moiety is a polynucleotide.
78. A method according to claim 76, wherein the mixture of step (c) comprises at least two deoxynucleotides each comprising a different base.
79. A method according to claim 76, wherein the primer hybridizes to the target nucleic acid immediately adjacent the specific nucleotide being identified.
80. A method according to claim 76, wherein the detectable amount of target nucleic acid polymer is obtained by performing an amplification reaction.

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81. A method according to claim 76, wherein the one or more deoxynucleotides are deoxynucleoside triphosphates selected from the group consisting of dATP, dGTP, dCTP and dTTP.
82. A method according to claim 76, wherein the chain terminating nucleotide analogue is selected from group consisting of ddATP, ddGTP, ddCTP and ddTTP.
83. A method according to claim 76, wherein the polymerization agent is a DNA polymerase.
84. A method according to claim 76, wherein the primer extension product is removed from the target nucleic acid prior to analysis.
85. A method according to claim 76 or 77, wherein a plurality of specific nucleotides may be detected at different defined sites in one or more target nucleic acid polymers by hybridizing a plurality of oligonucleotide primers to complementary sequences in the one or more target nucleic acid polymers flanking each site and extending the primers to form distinguishable extended primers.
86. A method according to claim 76, wherein the target nucleic acid is obtained or derived from the cells of a patient and the detection of the specific nucleotide indicates predisposition to a genetic disease in the patient.
87. A method for determining the identity of a specific nucleotide at a defined sites in a target nucleic acid polymer, comprising the steps of:
- (a) providing a detectable amount of a target nucleic acid polymer in a single stranded form;

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(b) hybridizing the detectable amount of the nucleic acid polymer with an oligonucleotide primer, wherein the primer has a nucleotide sequence that is complementary to a sequence in the target nucleic acid polymer, such that when the primer is hybridized to the target nucleic acid polymer the 3' end of the primer binds to a nucleotide flanking the specific nucleotide at the defined site in the target nucleic acid;

(c) exposing the hybridized nucleic acid polymer to a polymerization agent in a mixture containing at least one deoxynucleotide and a chain terminating nucleotide analogue, such that a detectable primer extension product is formed which differs depending upon whether the chain terminating nucleotide analogue is complementary or not complementary to the defined site, and

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(d) analyzing the polymerization mixture of step (c) for the primer extension product, whereby the identity of the specific nucleotide at the defined site is determined.

88. A method according to claim 87, wherein the mixture of step (c) comprises at least two deoxynucleotides each comprising a different base.

89. A method according to claim 87, wherein the primer hybridizes to the target nucleic acid immediately adjacent the specific nucleotide being identified.

90. A method according to claim 87, wherein the detectable amount of target nucleic acid polymer is obtained by performing an amplification reaction.

91. A method according to claim 87, wherein the one or more deoxynucleotides are deoxynucleoside triphosphates selected from the group consisting of dATP, dGTP, dCTP and dTTP.

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92. A method according to claim 87, wherein the chain terminating nucleotide analogue is selected from group consisting of ddATP, ddGTP, ddCTP and ddTTP.
93. A method according to claim 87, wherein the polymerization agent is a DNA polymerase.
94. A method according to claim 87, wherein the primer-extension product is removed from the target nucleic acid prior to analysis.
95. A method according to claim 87 or 88, wherein a plurality of specific nucleotides may be detected at different defined sites in one or more target nucleic acid polymers by hybridizing a plurality of oligonucleotide primers to complementary sequences in the one or more target nucleic acid polymers flanking each site and extending the primers to form distinguishable extended primers.
96. A method according to claim 87, wherein the target nucleic acid is obtained or derived from the cells of a patient and the detection of the specific nucleotide indicates predisposition to a genetic disease in the patient.

REMARKS

This application is a continuation of U.S. application serial No. 08/162,376 which has now issued as U.S. Patent No. 6,013,431.

Claim 51 has been amended to recite at "least one deoxynucleotide and at least one chain terminating nucleotide analogue." Support for this amendment can be found throughout the specification and for example at page 18, lines 23 to 29 through page 19, lines 1-2.